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Curcuminoid Prevents Protein Oxidation but not Lipid Peroxidation in Exercise Induced Muscle Damage Mouse

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Abstract

Oxidative stress is believed as underlined mechanism of exercise induced muscle damage. This study was aimed to investigate curcuminoid effect on protein oxidation and lipid peroxidation muscle of exercised induced model. Adult male healthy mice were used as experiment models, grouped in to curcuminoid treated, placebo (corn oil only treated) and untreated group. Protein carbonyl level was significantly lower in curcuminoid treated group compared with untreated group ($p = 0.003$). In the contrary, the malondialdehyde level was not significantly different between those groups ($p = 0.092$).

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Keywords: curcumin; malondialdehyde; protein carbonyl; eccentric; downhill

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Nomenclature

BW	Body weight
DOMS	Delayed Onset Muscular Soreness
GST	Glutathion s tranferase
kg	kilogram
MDA	Mallondialdehyde
mg	milligram
nmol	nano mol
sTnI	Skeletal muscle Troponin I

1. Introduction

Curcuminoid is active compound found in the *Curcuma longa L* extract, consisted of 95% curcumin, 5% *desmethoxy* and *bidesmethoxy curcumin*¹. Previous studies reported it antioxidant activity in protection of serious damage tissue, such as liver and kidney¹. Curcuminoid is a potent radical scavenger for reactive oxygen species (ROS) and it also stimulates some antioxidant endogen enzymes activity.

Delayed onset muscular soreness (DOMS) is a damage of muscle at late onset of recovery phase after exercise stop^{3,4}. Oxidative stress is believed as underlined mechanism of exercise induced muscle damage². Unfortunately, curcuminoid administered after exercise stop was failed to protect muscle damage². We look forward for this explanation. This study was aimed to investigate curcuminod effect on oxidative stress marker, such as mallondialdehyde and protein carbonyl level in the exercise induced muscle.

2. Methods**2.1. Materials**

Curcuminoid was obtained from standardized *Curcuma Longa L* extract of *NHK Laboratories Inc Cat. TUR011*. It was suspended in 0.4 ml corn oil for each 100 mg curcuminoid. Models were used male healthy 10 weeks balb/c mice, obtained from LPPT pre-clinical unit Gajahmada University. Mouse was trained downhill running on Columbus treadmill apparatus. Skeletal muscle damage marker was determined by measurement of sTnI level using ELISA Medicine kit. The MDA level was determined using thiobarbituric acid, obtained from Sigma Chemical. Protein carbonyl level was determined colorimetric assay using Oxiselect kit *Cat no STA-31*.

2.2. Methods

A day before exercise, curcuminoid was administered orally in to treated group by gauge 4 mg/kg BW in corn oil suspension. Untreated mice were given only corn oil as placebo. Muscle damage was obtained by downhill running on Columbus treadmill, 30 cm/s for 18 minutes with 5 minutes preconditioning. Mice were recovery for 4 hours after exercise stop, then anesthetised and harvest it blood and calf muscles. Blood was taken 1.5 ml from the heart of ketamine anesthetized mouse and process for it serum. Calf muscle were taken from both right and left legs and grind in the cold PBS solution to get the muscle homogenate. Level of sTnI was measured using ELISA sandwich method. Muscle homogenates were examined for mallondialdehyde and protein carbonyl level using colorimetric method. All procedures were approved for ethical clearance from Animal Care and Use Committee Faculty of Veterinary, Universitas Airlangga.

3. Results and discussion

The sTnI level of curcuminoid treated mouse was significantly lower compared with untreated group ($p = 0.003$) but it was not different with normal mice ($p = 0.165$). Curcuminoid protected muscle against excised induced damage in mice. Our result did not support previous study that reported curcuminoid failure in muscle damage

inhibition². We suggested it was due to the onset of curcuminoid supplementation. Protection effect of curcumin was resulted at the onset of early supplementation. The result of sTnI serum level measurement was showed at Table 1.

Table 1. The sTnI serum level comparasion

Group	N	Mean \pm SD (ng/ml)	Levene test (p)	ANOVA (p)
Normal	9	0.33 \pm 0.10 ^a	0.185	0.0001
Untreated	9	0.58 \pm 0.08 ^b		
Curcuminoid treated	9	0.41 \pm 0.14 ^a		

Note: different superscribe indicating significantly different of sTnI serum level ($p < 0.05$)

Curcuminoid was reported to be able to stimulate antioxidant response element of glutathion s transferase (GsT) expression^{2,4}. Glutathion is transfered and bound in to troponin I skeletal muscle, increased it affinity to the calcium and contraction response to the destructive eccentric force. Troponin I is myofilament which has oxidative target residue at cysteine no 133 for fast twitch and 134 for slow twitch characteristic⁶. Oxidized sTnI induced myofibril defragmentation and transported outward through cell membrane⁷. Glutathion acted as scavenger for sTnI and prevented sTnI oxidation⁶. It was suggested that protection effect of curcuminoid due to this glutathionylated sTnI mechanism.

In order to support this argument, we measured the protein carbonyl level of muscle. Protein carbonyl is residue produced from protein oxidation⁸, such as sTnI. Lower level of protein carbonyl was indicating the number of myofilament damage was also limited. Protein carbonyl level in curcuminoid treated muscle were significantly lower compared with the untreated ($p = 0.003$). The result of protein carbonyl measurement was showed at Table 2.

Table 2. The protein carbonyl level of muscle

Group	N	Mean \pm SD (nmol/mg muscle protein)	Levene test (p)	Brown Forsythe test (p)
Normal	9	31.89 \pm 13.05 ^a	0.014	0.0001
Untreated	9	72.05 \pm 27.28 ^b		
Curcuminoid treated	9	29.33 \pm 14.78 ^a		

Note: different superscribe indicating significantly different of protein carbonyl level ($p < 0.05$)

Unfortunately, the curcuminoid was not able to low the lipid peroxidation. The level of mallondialdehyde curcuminoid treated muscle was not different with untreated. ($p = 0,092$). It was indicating that protein oxidation was more closely related to the mechanism of muscle damage compared with lipid peroxidation. The inhibition of protein oxidation was better to prevent the muscle damage than lipid peroxidation. Further, we need to define a novel strategy for muscle damage prevention. The use of scavengger antioxidant was left behind because this is the era of substance that encourage our antioxidant endogen system.

Table 3. The mallondialdehyde level of muscle

Group	N	Mean \pm SD (nmol/mg muscle protein)	Levene test (p)	ANOVA (p)
Normal	9	35.66 \pm 11.09 ^a	0.569	0.0001
Untreated	9	67.86 \pm 14.74 ^b		
Curcuminoid treated	9	55.56 \pm 17.96 ^b		

Note: different superscribe indicating significantly different of mallondialdehyde level ($p < 0.05$)

4. Conclusion

Curcuminoid prevent protein oxidation but not lipid peroxidation. This is the underlined mechanism for muscle to maintain its structure and its function in response of eccentric force exposure.

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